Immunocytochemistry Followed by FISH (Version 3)

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***We present multiple versions of the Immunocytochemistry / FISH protocols because the conditions under which each antibody and DNA probe work can be different and must be determined emperically.**

Reagents

Acetic acid, glacial
Bovine Serum Albumin (BSA)
Boehringer Mannheim Biochemicals (BMB), Cat. 100 350
DAPI
BMB, Cat. 236 276
Formamide
FLUKA BioChemica, Cat. 47670
Goat anti-rabbit-TRITC (secondary)
Sigma, Cat. T-5268
HCl, 1 M
Methanol
Para-Formaldehyde
Sigma, Cat. P6148
Phosphate Buffered Saline, pH 7.4
Invitrogen Corp., Cat. 10010-023
Primary antibody
Specific for desired protein, made in either a mouse or rabbit
Rabbit anti-mouse-TRITC (secondary)
Sigma, Cat. T2402
NaOH, 0.1 M
20X SSC
Tween 20
Sigma, Cat. P1379
Vysis CEP® Probe
Vysis

Preparation

Methanol

1. Room temperature
2. Pre-chill to -20°C

Permeabilization Buffer

Triton X-100	50 μl	f.c. [0.53]
1X PBS	10 ml	

Blocking Solution (3% BSA/1X PBS)

BSA	0.3 g
1X PBS	10 ml

Store at 4˚C

Antibody Solution (1% BSA/1X PBS)

Blocking solution	300 µl
1X PBS	600 µl

2% p-formaldehyde

p-formaldehyde	2 g	
1X PBS	100 ml	
0.1 N NaOH	500 µl	f.c. [0.5 mM]

Adjust to pH 7.4 with HCl

Store <1 month at 4˚C

50% FA/SSC

20X SSC	20 ml
dH_2O	80 ml
Formamide	100 ml
Total	200 ml

Adjust pH to 7-7.5 with 1 M HCl

Pre-warm to 45˚C

DAPI (stock solution)

DAPI	2 mg	f.c. [0.2 mg/ml]
dH_2O	10 ml	

Aliquot and store at -80˚C

DAPI (staining solution)

DAPI stock solution	40 µl	f.c. [80 ng/ml]
2X SSC	100 ml	

Antifade (1,4-phenylene-diamine)
See Antifade preparation procedure in CGH Protocols

Procedure

1. Grow adherent cells in chamber slides.
2. Fix cells in methanol pre-chilled to -20˚C for 10 min at RT.
3. Wash 3 x 5 min 1X PBS at RT.
4. Permeabilize cells with 0.5% Triton X-100/PBS 5 min at RT.
5. Wash 3 x 5 min 1X PBS at RT.
6. Remove chamber and block slides with 120 µl blocking solution in hybridization chamber 30 min at 37˚C.
7. Incubate with 1˚ Ab (rabbit or mouse) in 120 µl antibody solution in hybridization chamber at 37˚C for 45 min.
8. Wash 3 x 5 min with 1X PBS at RT.
9. Incubate with 2˚ Ab [goat anti-rabbit-TRITC (1:200) or Rabbit anti-mouse-TRITC (1:200), respectively, in 120µl antibody solution] in hybridization chamber at 37˚C for 60 min.
10. Wash 3 x 5 min 1X PBS at RT.
11. Fix with methanol:acetic acid (3:1) at RT 10 min.
12. 2% p-formaldehyde at RT for 1 min.
13. 70%, 90%, 100% ethanol series (3 min each).

Note: Can counterstain with DAPI at this point and mount slides with antifade to have a look at them and determine if Ab detection worked. Wash coverslips 3 x 5 min in 2X SSC before continuing with procedure.

14. Combine 1 µl Vysis CEP® probe, 1 µl water, and 7 µl Vysis Hyb Buffer.

15. Add probe cocktail to slide, coverslip, and seal with rubber cement.

16. Denature slide 75˚C for 5 min on slide warmer.

17. Incubate in hybridization chamber at 37˚C overnight.

18. Remove rubber cement.

19. Wash in FA/SSC pre-warmed to 45˚C for 21 min, shaking.

20. Stain for 2 min with DAPI.

21. Wash in 2X SSC for 10 min, shaking.

22. Mount with antifade.